

WHAT IS CLAIMED IS:

1. A method for making a chimeric ungulate comprising:

(a) introducing an ungulate embryonic stem cell that has a first genetic complement into a recipient embryo of the same species as the embryonic stem cell, said recipient having a second genetic complement, to form a chimeric ungulate embryo; and

(b) placing the chimeric ungulate embryo in an environment suitable for the completion of development to form a chimeric ungulate.

2. The method of claim 1, wherein the ungulate embryonic stem cell is pluripotent.

3. The method of claim 2, wherein the ungulate embryonic stem cell is totipotent.

4. The method of claim 1, wherein the embryonic stem cell is introduced into the embryo at a pre-implantation stage.

5. The method of claim 4, wherein the pre-implantation stage is the blastocyst stage.

6. The method of claim 1, wherein the embryonic stem cell is derived from a first breed of ungulate and the recipient embryo is derived from a second breed of the same species as the first breed.

7. The method of claim 6, wherein the first breed and the second breed are swine.

8. The method of claim 7, wherein the first breed of swine is the Meishan breed, and the second breed is the Duroc breed.

9. The method of claim 1, wherein the first genetic complement is different from the second genetic complement.

10. The method of claim 9, wherein the first genetic complement comprises an exogenous nucleotide sequence stably integrated into the genetic complement of the embryonic stem cell.

11. The method of claim 10, wherein the first genetic complement comprises a nucleotide sequence capable of being expressed to provide human Factor IX in recoverable form from the chimeric ungulate.

12. The method of claim 10, wherein the first genetic complement comprises a nucleotide sequence encoding a protein selected from the group consisting of human blood proteins, human hormones, human growth factors, human cytokines, human enzymes, human hormone receptors, human binding proteins, antigens, translation factors, transcription factors, onco-proteins, protooncoproteins, human milk proteins, and human muscle proteins.

13. The method of claim 10, wherein the first genetic complement comprises a porcine nucleotide sequence encoding a protein selected from a group that improves porcine carcass composition, porcine carcass weight, porcine disease resistance and porcine milk production.

14. A chimeric ungulate produced according to the method of any one of claims 1 through 13.

15. A method of isolating and purifying an embryonic stem cell culture, said method comprising:

(a) preparing a first culture by culturing dissociated cells from an ungulate embryo in conditioned stem cell medium in the absence of a feeder layer; and

(b) subculturing the first culture until a second

stable culture with morphological features and growth parameters characteristic of an embryonic stem cell culture is established.

16. The method of claim 15, wherein the dissociated cells from an ungulate embryo are obtained from an ungulate embryo which was developed *in vitro* in stem cell medium (SCM) on a feeder layer.

17. The method of claim 15, wherein the stem cell medium is conditioned by Buffalo Rat Liver Cells, and includes growth factors, vitamins, amino acids and antibiotics.

18. The method of claim 17, wherein the stem cell conditioned medium (CSCM) comprises approximately 40% of stem cell medium (SCM) and approximately 60% of Buffalo Rat Liver Cell conditioned medium(BRL/CM) .

19. The method of claim 15, wherein the morphological features of cells isolated from the culture comprise a round shape, as observed with the light microscope, a diameter of approximately 8-15 microns, and a cytoplasmic to nuclear diameter ratio of approximately 10-25:75-90, and wherein the growth parameters of the cells in culture comprise a doubling time of approximately 18-36 hours and multilayered rather than monolayered growth.

20. The method of claim 15, further defined as producing an embryonic stem cell culture which comprises at least 50% of cells that are capable of forming a teratoma or a teratocarcinoma when introduced into a host mouse.

21. The method of claim 15, wherein the embryo is obtained from a swine of the Meishan line.

22. An embryonic ungulate stem cell isolated from a

culture made in accordance with claim 15.

23. A method of making a transgenic ungulate, said method comprising transferring a nucleus from the cell of claim 22 into an ungulate recipient cell from which an embryo develops.

24. The method of claim 23, wherein the recipient cell is an enucleated ungulate ovum.

25. The method of claim 23, wherein the recipient cell is an enucleated ungulate embryonic cell.

26. The isolated ungulate cell of claim 22 which is totipotent.

27. A culture initiated from the stem cell of claim 26.

28. A stable cell line derived by subculturing the culture of claim 27.

29. The embryonic ungulate stem cell of claim 22 which has a genetic complement comprising the complement of the ungulate source of the stem cell and an exogenous nucleotide sequence stably integrated into said complement.

30. The embryonic ungulate stem cell of claim 29, wherein the exogenous nucleotide sequence encodes a selectable marker.

31. The embryonic cell of claim 30, wherein the selectable marker comprises hygromycin (Hph), puromycin (Pac), neo, ada and dHFR.

32. A transgenic ungulate descended from a chimeric ungulate of claim 14.

33. A method of making an ungulate from which tissues can be used as a xenograft, said method comprising:

(a) incorporating the genetic complement from the embryonic ungulate stem cell of claim 22 into a host ungulate embryonic cell, to form a chimeric ungulate wherein said genetic complement renders tissue from a chimeric ungulate histocompatible with a recipient for the xenograft; and

(b) breeding the chimeric ungulate to form an offspring ungulate which includes the tissues for the xenograft.

34. The method of claim 33, wherein the offspring ungulate is a transgenic ungulate.

35. A method of using a transgenic ungulate of claim 32 to produce an exogenous protein, said transgenic ungulate having a genetic complement which comprises a nucleotide sequence capable of providing said exogenous protein, said method comprising exposing said ungulate to conditions wherein the nucleotide sequence is activated to provide said exogenous protein in a recoverable form in an ungulate body fluid or tissue, and recovering said protein from said body fluid or tissue.

36. The method of claim 35, wherein the body fluid is milk secreted from a female ungulate.

37. The method of claim 35, wherein the exogenous protein is selected from the group consisting of TNF α , human growth factor, a human peptide hormones, an ungulate growth factor, and an ungulate milk protein.

38. The method of claim 37, wherein the growth factor is EGF.

39. An isolated embryonic stem cell from an ungulate.

40. The embryonic stem cell of claim 39, wherein the cell is totipotent.

41. The embryonic stem cell of claim 39, which is negative when assayed for the presence of a structural protein which is only present in a differentiated cell.

42. The embryonic stem cell of claim 41, wherein the structural protein is cytokeratin 18 or vimentin.

43. The embryonic stem cell of claim 39, which is negative when assayed for the presence of an antigen which is only present in a differentiated cell.

44. The embryonic stem cell of claim 43, wherein the antigen is a neurofilament, a glial fibrillar acidic protein, keratin or desmin.

45. The embryonic stem cell of claim 44, wherein the neurofilament is a protein with a molecular weight of 68, 160 or 200 kd.

46. The embryonic stem cell of claim 39, which has a rounded shape as observed with the light microscope, a diameter of approximately 8-15 microns, and a cytoplasmic to nuclear diameter ratio of approximately 10-25: 75-90.

47. The embryonic stem cell of claim 39, which in a stable culture exhibits multilayered growth on a solid surface and a doubling time of approximately 18-36 hours.

48. The embryonic stem cell of claim 39, wherein the cell is capable of forming a teratoma or a teratocarcinoma when introduced into an immunodeficient host animal.

49. The embryonic stem cell of claim 39, wherein the source of the cell is an embryo of a swine line selected

from the group consisting of Meishan, Duroc and Yorkshire.

50. The embryonic stem cell of claim 39, wherein the cell is capable of producing a chimeric swine when said cell is introduced into a recipient embryo.

51. The embryonic stem cell of claim 50, wherein the recipient embryo is derived from a swine line selected from the group consisting of Meishan, Duroc and Yorkshire.

52. The embryonic stem cell of claim 39, wherein said cell has a genetic complement comprising the complement of the ungulate of the embryo source of the stem cell and an exogenous nucleotide sequence stably integrated into said complement.

53. The embryonic stem cell of claim 52, wherein the exogenous nucleotide sequence is capable of being expressed to provide a protein in recoverable form from a host ungulate which comprises in its genetic complement, the complement of the embryonic stem cell.

54. The embryonic stem cell of claim 52, wherein the exogenous nucleotide sequence encodes a protein selected from the group consisting of blood proteins, hormones, growth factors, immune system regulatory factors, cytokines, enzymes, hormone receptors, binding proteins, antigens, translation factors, transcription factors, onco-proteins, protooncoproteins, milk proteins and muscle proteins.

55. The embryonic stem cell of claim 54, wherein the nucleotide sequence encodes a human protein.

56. A cell line initiated from the embryonic stem cell of claim 39.

57. A stable cell line initiated from a culture of embryonic stem cells.

58. The cell line of claim 57 which is a clonal line.

59. The cell line of claim 57 which grows in a multilayer rather than a monolayer when cultured on a solid surface and has a doubling time of approximately 18-36 hours.

60. The cell line of claim 57, wherein a cell isolated from the line has a round shape, as observed with the light microscope, a diameter of approximately 8-15 microns, and a cytoplasmic to nuclear diameter ratio of approximately 10-25: 75-90.

61. The cell line of claim 57, which is negative when assayed for the presence of a structural protein which is only present in a differentiated cell.

62. The cell line of claim 61, wherein the structural protein is cytokeratin 18 or vimentin.

63. The cell line of claim 57, which is negative when assayed for the presence of an antigen which is only present in a differentiated cell.

64. The cell line of claim 63, wherein the antigen is a neurofilament, a glial fibrillar acidic protein, keratin or desmin.

65. The cell line of claim 64, wherein the neurofilament is a protein with a molecular weight of 68, 160 or 200 kd.

66. The cell line of claim 56 designated D195.

67. The cell line of claim 56 designated M1192.

68. An embryo comprising the cell of claim 39.

69. A clone of swine embryos derived from the embryo of claim 68.

70. A chimeric swine embryo comprising a cell derived from a swine embryonic stem cell.

71. An embryo of an ungulate made by the process comprising transfer of a nucleus of an isolated embryonic stem cell into a recipient cell from the same species as the embryonic stem cell.

72. The embryo of claim 71, wherein the recipient cell is an enucleated embryonic cell.

73. The embryo of claim 71, wherein the recipient cell is an enucleated ovum.

74. An embryo cloned from the embryo of claim 71.

75. An embryo of claim 68 or 71, which comprises an exogenous nucleotide sequence stably integrated into its genetic complement.

76. An isolated nucleus of an embryonic stem cell from an ungulate.

77. Progeny of the chimeric ungulate of claim 14.